

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Cardiac impairment in rabbits fed a high-fat diet is counteracted by dehydroepiandrosterone supplementation

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/128573> since

Published version:

DOI:10.1016/j.lfs.2009.04.020

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)



UNIVERSITÀ DEGLI STUDI DI TORINO

This Accepted Author Manuscript (AAM) is copyrighted and published by Elsevier. It is posted here by agreement between Elsevier and the University of Turin. Changes resulting from the publishing process - such as editing, corrections, structural formatting, and other quality control mechanisms - may not be reflected in this version of the text. The definitive version of the text was subsequently published in *Life Sciences*, Volume 85, Issues 1–2, 1 July 2009, Pages 77–84, <http://dx.doi.org/10.1016/j.lfs.2009.04.020>

You may download, copy and otherwise use the AAM for non-commercial purposes provided that your license is limited by the following restrictions:

- (1) You may use this AAM for non-commercial purposes only under the terms of the CC-BY-NC-ND license.
- (2) The integrity of the work and identification of the author, copyright owner, and publisher must be preserved in any copy.
- (3) You must attribute this AAM in the following format: Creative Commons BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/deed.en>), <http://dx.doi.org/10.1016/j.lfs.2009.04.020>

Cardiac impairment induced by oxidative stress in rabbits fed a high-fat diet is counteracted by dehydroepiandrosterone supplementation

Manuela Aragno ¹, Giorgia Meineri, Ilenia Vercellinato ¹, Paola Bardini ¹, Stefania Raimondo ⁵,
Pier Giorgio Peiretti ³, Andrea Vercelli ⁴, Giuseppe Alloatti ⁶, Chiara Elena Tomasinelli ¹, Olivero
Danni ¹, Giuseppe Boccuzzi ⁷.

¹Department of Experimental Medicine and Oncology, University of Turin - Corso Raffaello 30, 10125 Turin, Italy; ²Department of Animal Production, Epidemiology and Ecology, University of Turin - Via L. da Vinci 44, 10095 Grugliasco, Italy; ³Institute of Food Science and Production, IZPA, - Via L. da Vinci 44, 10095 Grugliasco, Italy; ⁴Associated Veterinary Ambulatory, Corso Traiano 99, 10135, Turin, Italy; ⁵Department of Clinical and Biological Sciences, University of Turin - Regione Gonzole 10, San Luigi Hospital, 10043 Orbassano, Italy; ⁶Department of Animal and Human Biology, University of Turin - Via Accademia Albertina 13, 10123 Torino; ⁷Department of Clinical Pathophysiology, University of Turin - Via Genova 3, 10126 Turin, Italy;

Running head Cardiomyopathy in rabbit fed a high-fat diet

Corresponding author: Manuela Aragno, Department of Experimental Medicine and Oncology, University of Turin - Corso Raffaello 30, 10125 Turin, Italy.

phone: 0039-0116707758, fax: 0039-0116707753, e-mail: manuela.aragno@unito.it

Supported by Regione Piemonte, MIUR (Turin University and COFIN) and CRT Foundation (Alfieri Project-Cardiomiopatia diabetica: individuazione di nuove strategie terapeutiche).

Authors disclosures: M. Aragno, G. Meineri, L. Vercellinato, P. Bardini, S. Raimondo, PG. Peiretti, A. Vercelli, G. Alloatti, C. E. Tomasini, O. Danni, G. Boccuzzi, no conflicts of interest.

Abstract

The role of oxidative stress in the activating of downstream signalling leading to structural and functional changes in the left ventricle of rabbits fed a high-fat diet for 12 wks and the effect of dietary dehydroepiandrosterone supplementation, were analyzed. The animals showed hyperglycemia, insulin-resistance, dyslipidemia and early features of cardiomyopathy, i.e. altered myosin heavy-chain isoforms, tissue degeneration and reduced contractility of the papillary muscle. Dietary supplementation with dehydroepiandrosterone (0.02%,wt/wt) in these animals reduced oxidative stress evaluated as levels of reactive oxygen species and hydroxynonenal levels, without affecting high plasma glucose level or insulin resistance. Dehydroepiandrosterone supplementation also counteracted both the activation of redox-sensitive transcription factor and of nuclear transcription factor- κ B, and the expression of tumor necrosis factor- α and of C-reactive protein-mRNA. Improvement of the oxidative balance counteracted the shift to myosin heavy chain isoforms, ameliorating the heart contractility. A high-fat diet induced oxidative stress and metabolic syndrome in this rabbit model, and that dehydroepiandrosterone, by restoring oxidative balance, lowering lipid levels and down-regulating inflammation, prevented molecular and functional alterations of the cardiac muscle, thus exerting a beneficial effect and delaying the onset of diabetic cardiomyopathy.

Introduction.

Diabetic cardiomyopathy is characterized by systolic and diastolic dysfunction and has been reported in diabetic patients with no ischemic, valvular or hypertensive heart disease. Its development includes metabolic disturbances, small-vessel diseases, autonomic dysfunction, insulin-resistance and myocardial fibrosis (1,2). Recently, an important role in its pathophysiology has been attributed to the generation of reactive oxygen species, which activates a number of secondary-messenger pathways, finally leading to cardiac dysfunction (3-5). We recently showed that free radical overproduction appears early in human type 2 diabetes (6) and that, in a rat model of type 1 diabetes, oxidative damage plays a key role in the early development of cardiomyopathy (7). Antioxidants might counteract insulin resistance associated with type 2 diabetes and cardiovascular diseases (8-10). We reported elsewhere that in streptozotocin rats, the treatment with dehydroepiandrosterone (DHEA), a compound that possesses multi-targeted antioxidant properties (11-15), prevents myocardial damage induced by oxidative stress, while avoiding impairment of cardiac myogenic factors and the switch to myosin heavy chain expression (16). DHEA also counteracts oxidative imbalance and advanced glycation end-product formation in type II diabetic patients (7). The study targets diabetic cardiomyopathy, in a model of type 2 diabetes induced by a high-fat (HF) diet in rabbits, an animal species that is highly susceptible to cardiovascular damage (17). Data show that DHEA treatment counteracted biochemical changes in the left ventricle induced by oxidative stress, improving myocardial function.

Materials and methods

Experimental procedure.

Male New Zealand White rabbits, 15 weeks old (Harlan-Italy, Udine, Italy) weighing 3.0 -3.5 Kg were cared for in compliance with the Declaration of Helsinki as revised in 1983, the Italian Ministry of Health Guidelines (no. 86/609/EEC) and with the *Principles of Laboratory Animal Care* (NIH no. 85-23, revised 1985). The rabbits were acclimatized for 2 weeks prior to the experiment in a room with 12h light dark cycle, individually housed in stainless steel cages in a temperature- and humidity-

controlled room ($23 \pm 3^{\circ}\text{C}$, $50 \pm 5\%$) and fed 100 g per day of standard rabbit non-purified diet, composition of 100 g: 16.2 g crude protein, 3.0 g crude fat, 7.3 g crude ash, 14.8 g fiber, 12 g moisture, added mineral 2.1, 40U/kg vitamin E) with appropriate certificate from the Association of Official Agricultural Chemists, 1975, and relative procedure analyses (Laboratorio Dottori Piccioni, Gessate Milanese, Italy). Rabbits were randomly distributed into four groups. The control group (C) ($n=4$) received standard rabbit diet; the DHEA-alone treated group (C-D) ($n=4$) received the same diet supplemented with 0.02% DHEA (Sigma Aldrich, Milan, Italy); the high-fat group (HF) ($n=4$) received a high-fat diet, consisting of standard rabbit non purified diet, composition of 100g: 16.2 g crude protein, 7.3 g crude ash, 14.8 g fiber, 12 g moisture, added mineral 2.1, 40U/kg vitamin E) plus 10 g added fat (6.7g corn oil and 3.3 g lard) (18); the HF plus DHEA group (HF-D) ($n=4$) received the high-fat diet supplemented with 0.02% DHEA. Two days before being killed, the rabbits were fasted overnight and the glucose tolerance test was performed. The rabbits were killed at 3 mo from the start of the experiment, by aortic exsanguination after anesthetization with Zoletil 100 (Tiletamine-Zolazepam, Virbac, Carros, France). Blood was collected and plasma and serum were isolated. The heart was rapidly excised and weighed, and portions of left ventricle were taken to obtain nuclear and total extracts. Other heart portions were utilized for histological microscopy. The papillary muscles were immediately removed for functional parameter detection.

General parameters.

Body weight and length of rabbits were measured at time zero and prior to death. The body mass index (BMI) was calculated as body weight in kilograms divided by body length in meters, squared (kg/m^2).

Oral glucose tolerance test

After a fasting period of 12h, a 50% glucose solution was orally administered to the rabbits at 1.5 g/Kg. Blood samples were collected via the auricular artery before 15, 30, 45, 60, 90, 120 and 240 min after glucose loading. Glucose levels were tested using an Accu-Check Compact kit (Roche Diagnostics GmbH, Mannheim, Germany)

70 **Parameters in plasma.**

71 Triglyceride (TG), total cholesterol (Te), low density-lipoprotein (LDL)- and high-density-
72 lipoprotein (HDL)-cholesterol were determined by standard enzymatic procedures using reagents kits
73 (Hospitex Diagnostics, Florence, Italy). Aspartate aminotransferase (AST) and lactate dehydrogenase
74 (LDH) were determined using an enzymatic kit (DiaSys Diagnostic Systems GmbH, Holzheim,
75 Germany). Plasma insulin was measured with an ultrasensitive insulin enzyme-linked
76 immunosorbent assay kit from DRG Diagnostics (Marburg, Germany). Insulin sensitivity was
77 calculated using the homeostasis model assessment (HOMA): fasting glucose (mmol/L) x fasting
78 insulin (U/L)/22.5.

79 **Tissue extracts (cytosolic, nuclear and total extracts).**

80 Cytosolic and nuclear fractions from rabbit left ventricle were prepared by the Meldrum *et al.*
81 modified method (19). Briefly, left ventricle (100 mg) was homogenized at 10% (w/v) in a Potter
82 Elvehjem homogenizer (Wheaton Science Products, Millville, NJ, USA) using a homogenization
83 buffer. Homogenates were centrifuged at 1,000 g for 5 min at 4°C. Supernatants were removed and
84 centrifuged at 15,000 g at 4°C for 40 min to obtain cytosolic fraction. The pellets were resuspended
85 in extraction buffer and incubated on ice for 30 min for high-salt extraction, followed by
86 centrifugation at 15,000 g for 20 min at 4°C. The resulting supernatants containing nuclear proteins
87 were carefully removed and samples were stored at -80°C until use.

88 Total extract was obtained by homogenizing at 10% (w/v) directly with extraction buffer and were
89 centrifuged at 1,000 g for 5 min at 4°C. Supernatants (total extract) were stored at -80°C until use.

90 Protein content was determined using the Bradford assay (20).

91 **Oxidative biochemical parameters.**

92 Reactive oxygen species (ROS) were measured in total extracts using 2',7' -dichlorofluorescein
93 diacetate (DCFH-DA) as a probe (21). Reduced and oxidized glutathione content was measured in
94 cytosolic fractions by Owens's method (22). The difference between total glutathione and GSH
95 content represents the oxidized glutathione (GSSG) content (expressed as *fmol/mg* proteins.); the ratio

96 between GSSG content and GSH is considered a good parameter of antioxidant status. 4-
97 hydroxynonenal (HNE) was detected by an HPLC procedure. The extract sample was directly
98 injected for HPLC (Waters Assoc., Milford, MA, USA) using an RP-18 column (Merck, Darmstadt,
99 Germany). The mobile phase used was 42% acetonitrile/bidistilled water (v/v). Serial concentrations
100 of HNE (0.5-10 μ mol/L) were used to prepare a standard curve (23). Catalase activity was evaluated
101 in the cytosolic fraction following Aebi's method (24). Total superoxide dismutase (SOD) activity
102 was assayed by the method described by Flohè and Otting (25). Since the activity of xanthine-
103 oxidase may vary, sufficient enzyme was used to produce a rate of cytochrome c reduction of at least
104 0.025 absorbance units/min in the assay without SOD.

105 **Advanced glycation end-products (AGE) detection with HPLC-MS.**

106 Cytosol fractions were treated with 6 mol/L hydrochloric acid for 2 h at 40°C and then centrifuged
107 (1860 g) (26); only the supernatant was utilized. A Thermo-Finnigan Surveyor instrument (Thermo
108 Electron, Rodano, Milan, Italy) equipped with autosampler and PDA-UV 6000 LP detector was used.
109 Mass spectrometry analyses were performed using an LCQ Deca XP plus spectrometer, with
110 electrospray interface and ion trap as mass analyzer. The chromatographic separations were run on a
111 Varian Polaris C18-A column (150 x 2 mm, 3 μ m particle size) (Varian, Leinì, Turin, Italy). Flow
112 rate 200 μ L min⁻¹. Gradient mobile phase composition was adopted: 95/5 to 0/100 v/v 5 mmol/L
113 heptafluorobutanoic acid in water/methanol in 13 min. The LC column effluent was delivered to a
114 UV detector (200-400 nm) and then to the ion source, using nitrogen as sheath and auxiliary gas
115 (Claind Nitrogen Generator apparatus, Lenno, Como, Italy). The source voltage was set to 4.5 kV in
116 the positive mode. The heated capillary was maintained at 200°C. The acquisition method used had
117 previously been optimized in the tuning sections for pentosidine quasi-molecular ion (capillary,
118 magnetic lenses and collimating octapole voltages) to maximize sensitivity. Collision energy (CE)
119 was chosen to maintain about 10 % of the precursor ion. The tuning parameters adopted for the ESI
120 source were: source current 80.00 μ A, capillary voltage 3.00 V, tube lens offset 15 V; for ion optics,
121 multipole 1 offset -5.25 V, inter multipole lens voltage -16.00 V, multipole 2 offset -9.00 V. Mass

122 spectra were collected in tandem MS mode: MS² of (+) 379 *m/z* with 33 % CE in the range 100-400
123 *m/z*.

124 **Western blotting.**

125 NFκB-p65 on cytosol and nuclear extracts of left ventricle were detected by Laemmli's method (27).
126 Anti-α-actinin antibodies served as loading control for cytosolic proteins and anti-Lamin-B1 for
127 nuclear NFκB. Specific bands were quantified by densitometry using analytic software (Bio-Rad,
128 Multi-Analyst, München, Germany) and the net intensity of bands in each experiment was
129 normalized for the intensity of the corresponding α-actinin or lamin-B1 bands, before comparison
130 between control and treated samples.

131 **RNA isolation and RT-PCR .**

132 Total RNA was isolated using the RNA fast kit (Molecular Systems, San Diego, CA, USA). Total
133 DNA was amplified using sense and antisense primers specific for the C-reactive protein (CRP) gene
134 (sense 5'-AGGATCAGGATTCGTTTG-3' and antisense 5'-CACCACGTACTTGATATGTC-3'),
135 the tumor necrosis factor alpha (TNFα) gene (sense 5'-AGGAAGAGTCCCCAAACAACCT-3' and
136 antisense 5'-GGCCCGAGAAGCTGAT CTG), the myosin heavy-chain α (MHCα) gene (sense 5'-
137 GCCAAGGTGAAGGAGA TGAA-3' and antisense 5'-CTCTCCTGGGTCAGCTTCAG-3'), the
138 myosin heavy-chain β (MHCβ) gene (sense 5'-GGTCGAATACGTTACCATCTG-3' and antisense .
139 5'-AA TCGCTGTCCACAGTGGTCG-3') or for the glyceraldehyde-3-phosphate dehydrogenase
140 (GAPDH) gene (sense 5'-CGCCTGGAGAA AGCTGCTA-3' and antisense 5'-
141 CCCCAGCATCGAAGG TAGA-3').

142 The PCR reaction system contained 1 μL of RT product, 200 μmol/L dATP, dGTP, dCTP and dTTP
143 (Finnzymes, Espoo, Finland), 1.25 units of *Taq* DNA polymerase (Finnzymes, Espoo, Finland) and
144 50 pmol of sense and antisense primers in a total volume of 50 μL. All experiments were performed
145 on at least three independent cDNA preparations.

146 PCR products were electrophoresed on 2% agarose gels and amplification products were stained with
147 GelStar nucleic acid gel stain (FMC BioProducts, Rockland, ME, USA). Gels were photographed
148 and analyzed with Kodak 1D Image Analysis software. The net intensity of bands in each experiment

149 was normalized for the intensity of the corresponding GAPDHband before comparison between
150 control and treated samples.

151 **Histological staining.**

152 For standard histology, portions of left ventric1e were fixed in 4% neutralized formalin. Fixed
153 material was processed for hematoxylin and eosin staining. Six-micron paraffin-wax sections of left
154 ventric1e were used.

155 **Isolated papillary muscle and contractility determination.**

156 Papillary muscles were driven at constant frequency (120 beats/min) with a pair of electrodes
157 connected to a 302 T Anapulse Stimulator via a 305-R Stimulus Isolator (W.P. Instruments, New
158 Haven, CT, USA) operating in constant current mode. Isometric twitches were evaluated by a
159 Harvard transducer (60-2997), visualized on a Tektronix 2211 digital storage oscilloscope and
160 continuously acquired and recorded in a Power Mac computer, using Labview Software (Nationai
161 Instruments Corp., Austin, Texas, USA). The same software was used to measure developed peak
162 mechanical tension (T_{max}), maximum rate of rise and fall of developed mechanical tension ($+dT/dt$
163 $_{max}$ and $-dT/dt_{max}$), time-to-peak mechanical tension (ttp) and duration of contraction.

164 **Statistical analysis.**

165 Results are expressed as means \pm SD. ANOVA and post-hoc analyses included Bonferroni's test
166 were used (28). The SPSS 14.0 package for Windows (SPSS Inc., Chicago, IL) was used for the
167 statistical analysis. A value of $P < 0.05$ was viewed as statistically significant.

168 **Results.**

169 **General features.** Body weight, abdomen length, glucose and insulin were significantly increased in
170 rabbits fed a HP diet versus control values ($P < 0.05$) after three months' treatment. DHEA
171 supplementation did not modify these values (TABLE 1). Body mass index (BMI) and HOMA were

also significantly higher in HP rabbits than in controls ($P<0.05$) but not different from those of HF plus DHEA rabbits. The ratio heart/body weight ratio was the same in all groups, whereas the heart weight of rabbit fed the HF increased vs controls; DHEA supplementation reduced this increase. The results of the glucose tolerance test are in **FIG. 1**. After oral loading, glucose levels remained a high level for up to 240 min, in the HP and DHEA treated HF groups. TG, TC and plasma LDL-cholesterol concentrations were significantly higher in HF rabbits versus controls ($P<0.01$) and were lower in HF plus DHEA rabbits than in the HP alone group. ($P<0.05$). HDL-cholesterol was also significantly lower ($P<0.01$) in the HF group versus controls; in the HF plus DHEA group its value was similar to that of control animals ($P<0.05$).

Oxidative parameters in the left ventricle.

Rabbits fed a HP diet for 3 mo showed a significant increase in ROS levels in total extract of left ventricle vs the control group ($P<0.01$). 4-hydroxynonenal, an end-product of lipid peroxidation, also significantly increased in HP rabbits ($P<0.01$) (**FIGURE 2**). In rabbits fed with HF plus DHEA, the ROS and HNE levels were significantly lower than in the HP rabbits ($P<0.05$). Moreover, total SOD and catalase activities were also increased in the HP rabbits, and DHEA supplementation partially restored these activities to control levels. No significant difference in the GSSG/GSH ratio was observed among groups (data not shown). The level of pentosidine was significantly lower in the HF plus DHEA than in the HF alone group ($P<0.05$) (**TABLE 1**). In samples from both control and DHEA groups, gas-mass-HPLC analysis failed to detect any peak for pentosidine, apparently indicating its absence in these animals.

NFkB-p65.

Western Blot analysis detected NFkB-p65 protein in the nuclear and the cytosolic fractions of left ventricle (**FIGURE 3, panel A and panel B**). Nuclear p65-NFkB of HF rabbits was increased versus the control groups ($P<0.05$) (**panel A**) while p65-NFkB protein was reduced in the cytosolic fraction of HF rabbits ($P<0.05$) (**panel B**). In the rabbits fed the HF plus DHEA diet, cytosolic p65 protein content was less markedly reduced than it was in the HF group, corresponding to an increase

198 of the nuclear level of p65, and thus indicating lower activation of the NF κ B transcription factor
199 ($P<0.05$).

200 Inflammatory parameters.

201 Both indices of the proinflammatory state, TNF- α and CRP, were significantly increased in the heart
202 of rabbits fed the HF diet ($P<0.01$) (TNF- α : FIGURE 3, panel C and CRP: FIGURE 3, panel D).

203 The HF plus DHEA diet significantly decreased expression of both TNF- α and CRP, vs the HF
204 group ($P<0.05$).

205 Myosin expression.

206 PCR analysis was used to evaluate expression of two isoforms (α and β) of the myosin heavy-chain
207 (MHC) protein (FIGURE 4) in the left ventricle of control, DHEA, HF and HF plus DHEA rabbits.

208 The HF diet determined a significantly ($P<0.01$) decreased expression of α -MHC and an increased
209 expression of β -MHC. When DHEA was added to the HF diet, α -MHC was brought closer to the
210 control value, and β -MHC was reduced versus the control value ($P<0.05$).

211 Necrosis markers

212 LDH and AST release were evaluated in the plasma (FIGURE 5). Both LDH (panel A) and AST
213 (panel B) significantly increased in HF rabbits ($P<0.01$). In the HF plus DHEA rabbits, the levels of
214 LDH and AST were significantly lower than in the HF alone rabbits ($P<0.05$).

215 Histological analysis.

216 In the left ventricle of the HF rabbits, histological preparations clearly showed extensive and diffuse
217 lipid deposition (FIGURE 6, panel C). This lipid infiltration was not observable in either the control
218 or the DHEA alone group (panel A and panel B). Tissues obtained from HF plus DHEA rabbits
219 (panel D) showed rare areas of slight lipid deposition.

220 Cardiac function.

221 The contractile force developed by electrically-driven papillary muscles was evaluated in basal
222 conditions (TABLE 2). Basal contractility was weaker in papillary muscles from HF rabbits versus

controls; this was evident not only for maximal developed mechanical tension (T_{\max} : $P < 0.01$ by ANOVA), but also for maximum rate of rise ($+dT/dt_{\max}$; $P < 0.01$) and maximum rate of fall of developed mechanical tension ($-dT/dt_{\max}$; $P < 0.01$). In contrast, no significant difference was found between papillary muscles from control and HF rabbits in regard to time to peak mechanical tension (TPT) or duration of contraction. Treatment with DHEA significantly reduced the effects of the HF diet ($P < 0.05$). However, DHEA did not per se affect contractile properties of the papillary muscles.

Discussion

Male New Zealand rabbits fed a HF diet for 3 mo developed abdominal obesity, hyperglycemia, insulin-resistance, dyslipidemia, and cardiac dysfunction. DHEA supplementation did not affect the high plasma glucose levels induced by the HF diet, nor did it protect against hyperinsulinemia or the HOMA index. There is conflicting evidence about the effect of supplemental DHEA on glucose metabolism and insulin-sensitivity in healthy human subjects (29,30): our results are in agreement with several human studies showing only slight or no effect of DHEA on glucose homeostasis (31-33). As far as the plasma lipid profile is concerned, when DHEA was added to the HF diet, HDL increased in the plasma while triglycerides, total cholesterol and LDL were reduced. These results are in agreement with studies reporting that DHEA decreases serum triglycerides and the body weight in hyperlipidemic rats and diabetic mice (34,35). The mechanisms whereby DHEA exerts its anti-lipid effects are incompletely understood. However, it has been reported that the anti-obesity effect of DHEA may in part be related to changes in lipase activity and in beta-adrenergic receptor density (36,37). Moreover, DHEA accelerates lipid catabolism by direct regulation of hepatic lipid metabolism (38) and also has antiglucocorticoid activity, caused by direct modulation of the hepatic glucocorticoid receptor (39). However, alongside its effects on lipid metabolism and on insulin axis, we believe that DHEA's key action is against oxidative imbalance (6,7). Indeed, DHEA counteracted the increase in glycoxidative products observed in the plasma of rabbits fed a HF diet, in agreement with a previous rat study (13-16). Oxidative stress was also observed in the cardiac tissue of HF rabbits, in which there were

significant increases in ROS, end-products of lipid peroxidation (HNE) and activation of transcription factor NFkB. Activation of NFkB, which triggers in the inflammatory cascade, was demonstrated by the increased expression of TNF -alpha and CRP in cardiac tissue. Here we show that DHEA, by decreasing oxidative stress and reducing activation of NFkB transcription factor; may could determine a reduced expression of TNF and of CRP. Moreover, the anti-inflammatory effects of DHEA, due to cytokine reduction caused by decreased NFkB activation, might be amplified by the reduction of cholesterol levels, which in turn directly decreases CRP secretion from the tissues (40). CRP has been shown to predict morbidity and mortality from coronary heart disease (41). Moreover, it has been shown that high dietary cholesterol intake can increase the production of atherogenic inflammatory cytokines, such as IL-6 and TNF-a, and that reducing dietary cholesterol concentration leads to a reduction in CRP production (42).

We suggest that the pro-oxidant and inflammatory states, observed in HF rabbits, cause activation of intracellular signaling responsible for impaired myocardial function, as been reported in cases of heart failure (43). *HP* rabbits showed a switch of cardiac heavy-chain myosin from the alpha to the beta isoform: this event comprises the heart's "molecular motor" because contractile properties depend to a great extent on the isoform composition of MHC proteins. A switch in MHC isoform composition has been reported to cause reduced contractile velocity and energy expenditure (44). In man as in animals, a reduced content of α -MHC, which is expressed exclusively in the myocardium, has been reported to be responsible for the reduced myocardial contractility during heart failure (45) and in diabetes (46). In a previous model of type I diabetes cardiomyopathy (7), we reported that DHEA treatment, by avoiding impairment of cardiac myogenic factors and producing a switch in MHC gene expression, protected against tissue damage, an early event in diabetic cardiomyopathy. DHEA's modulatory effects on MHC expression were also observed in *HP* rabbits: expression of two isoforms, alpha and beta, in DHEA treated rats were not significantly different from those of the control group.

274 Rabbits on a HF diet clearly showed alterations in papillary muscle contractility. This was shown by
275 altered basal contractility, including reduced maximal developed tension, maximum rate of rise, and
276 maximum rate of fall of developed tension, which are signs of diastolic dysfunction. As in the case of
277 rat cardiac muscle (7), these alterations of the mechanisms controlling intracellular calcium handling
278 within cardiac myocytes are probably related to the structural damage caused by the HF diet.

279 Histological analysis of tissue from HF rabbits showed extensive and diffuse lipid deposition, as
280 reported in other animal models (47). The increased release of LDH and AST in the plasma of H.F.
281 rabbits confirmed myocardial damage. Treatment with DHEA protects the cardiac tissue from this
282 altered basal contractility, as well as minimizing histological changes and reducing cell damage
283 caused by the HF diet.

284 The role of DHEA in the cardiovascular system has been highlighted by the recent finding, in the
285 human heart, of DHEA production and CYP-17 gene expression, a key factor in DHEA synthesis
286 (48,49) and it has recently been proposed that the vascular protective effect of DHEA might be
287 dependent on G-protein-mediated activation of the phosphatidylinositol 3-kinase/ Akt
288 signaling pathway (50). Several explanations have been put forth for the multi-targeted antioxidant
289 effects of DHEA, including its effect on catalase expression (51), and its up-regulation of the
290 thioredoxin system (52), of the fatty-acid composition of cellular membranes and of cytokine
291 production. However, the precise mechanisms remain to be clarified, and additional non-antioxidant
292 effects cannot yet be ruled out. Whether the effect of DHEA is due to DHEA itself, to its metabolites,
293 or to a combination of both remains unclear. DHEA is also considered to be a pre-hormone, and it
294 has been speculated that, through hormonal effects it might modulate several metabolic pathways
295 which have nothing to do with antioxidant function. However, we found negligible variations of
296 either 17 β -estradiol or testosterone concentrations in rats treated with 4 mg DHEA. Nevertheless,
297 we report elsewhere that DHEA, but not a variety of other steroids including 17 β -estradiol, androstenedione and
298 dihydrotestosterone, protects bovine retinal capillary pericytes against glucose-induced lipid
299 peroxidation (53).

300 In conclusion, we show that DHEA supplementation can prevent molecular and functional alterations
301 of the cardiac muscle, restoring oxidative balance and lowering lipid levels, in rabbit fed a high-fat
302 diet. Since similar results have been reported in a model of diabetes type I diabetes, we suggest that
303 heart damage is chiefly dependent on impaired glucose metabolism, and not on insulin-resistance.
304 These data, together with our recent observations on type II diabetes patients (6), suggest that DHEA
305 treatment might prevent many events that lead to the cellular damage induced by hyperglycemia,
306 thus delaying the onset or progression of cardiac complications in type II diabetes.